The Veliger 30(3):291-294 (January 4, 1988)

THE VELIGER

Effect of Eyestalk Ablation on Oviposition in the Snail *Lymnaea acuminata*

by

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Abstract. The effects of eyestalk ablation on spawning in normal as well as cyclophosphamide-injected *Lymnaea acuminata* are reported. Unilateral or bilateral ablation of the eyestalk induced a spurt of spawning in snails. A second spurt of spawning could be induced in unilaterally ablated snails when the eyestalk of the other side was removed. Administration of cyclophosphamide, on the other hand, considerably reduced spawning. Eyestalk ablation, however, even in cyclophosphamide treated snails, stimulated egg laying to a considerable extent. It is proposed that, whereas the effect of cyclophosphamide is directly on the gonads, eyestalk ablation induces spawning through an endocrine mechanism.

INTRODUCTION

Information on the physiology of reproduction in pulmonate snails is fairly extensive; among these, the freshwater snail *Lymnaea stagnalis* (Basommatophora) has been studied in detail. The endocrine control of reproduction in the Basommatophora has been reviewed by JOOSSE & GERAERTS (1983) and GERAERTS & JOOSSE (1984). According to these authors, separate mechanisms regulate male and female systems in the hermaphrodite snail *L. stagnalis*. Specific neurohormones for the control of ovulation and the preparation of the egg mass have been suggested in these snails. In the stylommatophorans, optic tentacles are a source of an androgenic factor (RUNHAM, 1983) which helps in the differentiation of male sex cells. SINGH & AGARWAL (1981, 1983) demonstrated that injection of cyclophosphamide caused sterility in another hermaphrodite snail, *L. acuminata*.

In the present study the effect of eyestalk ablation on ovulation was studied in *Lymnaea acuminata*. Investigations were carried out on normal as well as on snails made sterile by the injection of cyclophosphamide (SINGH & AGARWAL, 1981, 1983). These studies have practical significance in that this snail is the intermediate host of the parasites *Fasciola gigantica* and *F. hepatica* which cause endemic fascioliasis in sheep and cattle.

MATERIALS AND METHODS

Adults of *Lymnaea acuminata* (2.6 ± 0.3 cm length) were collected from local freshwater ponds and kept in glass aquaria for 24 h in order to acclimatize them to laboratory conditions. Thereafter, the effect of eyestalk ablation on egg laying was studied in normal and cyclophosphamide injected snails. Groups of 20 snails were kept in 10-L capacity glass aquaria containing 3 L of dechlorinated tap water.

Egg masses of *Lymnaea acuminata*, which are laid in the form of gelatinous ribbons consisting of 5–120 eggs each, were collected every 24 h from the aquaria and transferred to 10-cm diameter petri dishes for counting the number of eggs. For ablation, the animals were gently picked out of an aquarium and either one or both eyestalks were quickly snipped off with a pair of iris scissors. Animals were then returned to the aquarium. Controls were likewise sham-operated on the foot.

Solutions of the desired strength of cyclophosphamide were prepared in distilled water and 50 μL was injected in the foot of the snails with an "Agala" micrometer syringe (SINGH & AGARWAL, 1981). Controls received distilled water alone.

Every experiment was conducted for six days on five groups of 20 snails each. Group A consisted of untreated controls; group B contained sham-operated controls kept in total darkness; group C consisted of eyestalk-ablated snails; group D consisted of ablated snails that were given 7 μg cyclophosphamide/snail daily for the first three days; group E received 7 μg of cyclophosphamide/snail daily for the first three days, following which their eyestalks were ablated.

Two sets of experiments were also conducted to study the effect of unilateral ablation. In one set, the left eyestalk was ablated and in the other set the right eyestalk was

Table 1

Effect of sham-operation, absence of light, and eyestalk ablation on egg laying in the snail *Lymnaea acuminata*. Table shows number of spawns and number of eggs laid by groups of 20 snails. Each value represents mean ± SE of 6 replicates with 20 snails in each replicate. Student's *t*-test were applied between the control and ablated snails to locate significant differences.

	Control (group A)		Sham-operated (kept in darkness) (group B)		Bilaterally ablated (group C)	
Period	Number of spawns	Number of eggs	Number of spawns	Number of eggs	Number of spawns	Number of eggs
24 h	7.33 ± 0.23	150.66 ± 5.37	5.66 ± 0.36*	131.5 ± 7.57	18.83 ± 0.91*	532.5 ± 29.12*
48 h	5.83 ± 0.18	148.66 ± 3.32	6.00 ± 0.40	129.66 ± 8.47	6.5 ± 0.54	154.5 ± 8.34
72 h	7.00 ± 0.28	131.32 ± 1.25	5.5 ± 0.37*	126.00 ± 5.78	3.83 ± 0.33*	92.83 ± 5.39*
96 h	6.33 ± 0.23	126.5 ± 4.77	5.5 ± 0.37	137.33 ± 7.13	2.5 ± 0.37*	41.33 ± 8.77*
120 h	6.33 ± 0.23	143.83 ± 9.31	5.33 ± 0.36	135.5 ± 8.68	1.66 ± 0.36*	17.66 ± 4.06*
144 h	6.16 ± 0.18	140.00 ± 3.29	5.33 ± 0.46	132.16 ± 3.36	0	0
Total number of eggs in 144 h		838		790		836

* Significantly ($P < 0.05$) different from control of corresponding period.

ablated. Controls were sham-operated as before. The eggs laid in both sets of unilateral ablation experiments were observed for 72 h. After this the eyestalk of the other side was also ablated and egg laying was studied for the next 72 h.

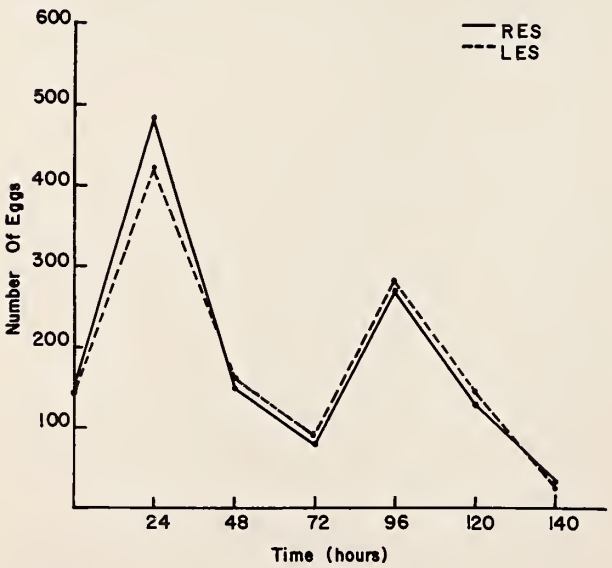

Figure 1

Graph showing effect of eyestalk ablation on pattern of egg laying in *Lymnaea acuminata* during 144-h period. Eggs were counted every 24 h. Data are mean of 6 replicates with 20 snails in each replicate. Zero time indicates number of eggs laid during the 24 h preceding ablation. Eyestalk of one side was removed and eggs were counted for the next 72 h; this was followed by removal of the eyestalk of the other side, with eggs counted for the subsequent 72 h. LES: initially left eyestalk was ablated, 72 h after which right eyestalk was ablated. RES: initially right eyestalk was ablated, 72 h after which left eyestalk was ablated.

Every experiment was replicated six times. Student's *t*-tests were applied to detect significant ($P < 0.05$) changes.

RESULTS

Groups of 20 normal *Lymnaea acuminata* together laid approximately 140 eggs/day during the six day observation period. An average of approximately 6 snails out of the group of 20 spawned every day (Table 1), indicating that over the entire observation period of 144 h each snail spawned approximately twice. The number of eggs laid by sham-operated controls kept in darkness did not differ significantly from non-operated controls kept in lighted aquaria (Table 1).

Bilateral removal of the eyestalks resulted in a sudden spurt of egg laying; during the first 24 h after eyestalk ablation an average of 18.83 spawns were laid. The number of eggs on the first day of ablation was 532 as compared to 150 in non-ablated snails (Table 1). This brisk rate of spawning, however, tapered off to no egg laying by the sixth day. The difference in the number of eggs between control and ablated snails was significant on five of six days ($P < 0.05$; *t*-test). The total number of eggs, however, laid by 20 snails in 144 h was 836 in the case of ablated and 838 in the case of non-ablated snails.

With unilateral eyestalk ablation also, there was a spurt of egg laying immediately after one of the eyestalks was removed. This started tapering off by the third day (Figure 1). Removal of the eyestalk on the other side, after 72 h, started a second spurt of egg laying (Figure 1). Thus, when the right eyestalk was removed 481 eggs were laid on the first day, 151 on the second, and 78 on the third. Removal of the other eyestalk resulted, after 24 h, in the laying of 273 eggs on the fourth day (Figure 1). Figure 1 also shows that right or left eyestalk ablation had the same effect on egg laying.

Table 2

Effect of cyclophosphamide treatment (7 μg/animal/day) and eyestalk ablation on egg laying in *Lymnaea acuminata*. Values are mean ± SE of 6 replicates of 20 snails each. All snails were injected with cyclophosphamide at 7 μg/day/animal for the first 3 days. Group D was ablated at the start of the experiment. Group E was ablated after 72 h. Student's *t*-test were applied to locate significant differences.

	Ablated on 1st day (cyclophosphamide injected for the first 3 days) (group D)		Ablated after 72 h (cyclophosphamide injected for the first 3 days) (group E)	
Period	Number of spawns	Number of eggs	Number of spawns	Number of eggs
24 h	8.16 ± 0.35	177.16 ± 8.98†	4.16 ± 0.18*,†	72.33 ± 1.80*,†
48 h	4.5 ± 0.24†	88.5 ± 2.93†	2.33 ± 0.23*,†	43.33 ± 1.95*,†
72 h	3.16 ± 0.33†	53.33 ± 6.63†	2.16 ± 0.33*	22.16 ± 4.54*,†
			(ABLATION)	
96 h	0	0	8.16 ± 0.44*,†	170.16 ± 5.13*,†
120 h	0	0	4.00 ± 0.28*,†	86.33 ± 3.07*,†
144 h	0	0	0	0

* Group E significantly ($P < 0.05$) different from group D.
† Significantly different from controls (Table 1).

Injection of cyclophosphamide at a dose of 7 μg/day for three days significantly ($P < 0.05$) reduced the number of eggs (Table 2). Thus, when cyclophosphamide was injected into non-ablated snails, the total number of eggs produced by 20 snails during the first three days was 137 as compared to 429 in control snails (Tables 1, 2). Injection of cyclophosphamide into ablated snails also reduced the number of eggs, but the reduction was significantly less than that observed in non-ablated snails during the first three days. In cyclophosphamide injected snails, however, oviposition ceased after three days (Table 2).

Eyestalk ablation, even in snails treated with cyclophosphamide for the first three days, resulted in a second spurt of egg laying for two days (Table 2). However, these eggs did not develop into young.

DISCUSSION

The present study clearly shows that ablation of the eyestalk(s) of *Lymnaea acuminata* initiates vigorous spawning activity within 24 h. In the beginning, the rate of oviposition was nearly 3.5 times higher than controls. From the number of spawns, eyestalk ablation apparently caused immediate spawning in nearly all of the snails. The rate of egg laying, however, gradually declined so that in the operated snails oviposition stopped completely after 120 h, even though the control snails continued to lay approximately the same number of eggs throughout the observation period. The present study also demonstrates that, even though eyestalk ablation changed the pattern of egg laying, the total number of eggs laid during the six day observation period was the same in both groups. Indeed, eyestalk ablation, although it causes a sharp increase in the rate of delivery of eggs immediately after ablation, did not cause any net increase in the number of eggs laid.

Data presented in this study shows that there was no change in the egg-laying pattern of sham-operated snails kept in total darkness. This rules out the possibility of blindness or injury being the cause of the egg-laying stimulus. Indeed, our study on unilateral ablation shows that removal of only one eyestalk of either side can cause increased egg laying. Moreover, a second spurt of spawning could be successfully started by the removal of the other eyestalk.

Singh & Agarwal (1981, 1983) demonstrated that the alkylating drug cyclophosphamide is a potent chemosterilant for *Lymnaea acuminata*. The present data show that ablation of eyestalks even in cyclophosphamide-treated snails caused a two-day spurt of egg laying. Since ablation can induce egg laying even in cyclophosphamide treated snails it is possible that ablation and cyclophosphamide act at different sites. Singh & Agarwal (1981, 1983) reported that cyclophosphamide reduced the DNA and RNA levels in the ovotestis of *L. acuminata*, thus indicating that the drug acts directly on gonads. It seems that ablation, which increases spawning in normal as well as in cyclophosphamide-injected snails, does not affect the gonads directly but through the neurohumoral system. Increased oviposition following ablation, three days after cyclophosphamide injection, also suggests that eyestalk removal can trigger the neurohumoral system even in sterile snails. There are a number of reports (Maat *et al.*, 1983; Scheerboom, 1978; Joosse & Veld, 1972; Bohlken & Joosse, 1982) that the caudo-dorsal cells in *L. stagnalis* release an ovulation hormone. It is possible that removal of the eyestalks in *L. acuminata* also stimulates the caudo-dorsal cells to release this hormone.

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